

- Symp. on Comparative Embryology of Angiosperms. Indian National Science Academy, New Delhi.
- . 1970b. Passifloraceae. Pp. 199–204 in Proc. Symp. on Comparative Embryology of Angiosperms. Indian National Science Academy, New Delhi.
- . 1970c. Caricaceae. Pp. 208–211 in Proc. Symp. on Comparative Embryology of Angiosperms. Indian National Science Academy, New Delhi.
- . 1970d. Cucurbitaceae. Pp. 212–219 in Proc. Symp. on Comparative Embryology of Angiosperms. Indian National Science Academy, New Delhi.
- SINGH, R. P. 1954. Structure and development of seeds in Euphorbiaceae. *Ricinus communis* L. Phytomorphology 4: 118–123.
- . 1956. Development of endosperm and embryo in *Phyllanthus niruri* L. Agra Univ. J. Res. (Sci.) 5: 163–167.
- . 1965. Structure and development of seeds in *Codiaeum variegatum* Blume. J. Indian Bot. Soc. 44: 205–210.
- . 1968. Structure and development of seeds in Euphorbiaceae: *Melanthesa rhamnoides* Wt. Beitr. Biol. Pflanzen. 45: 127–133.
- . 1969. Structure and development of seeds in *Euphorbia helioscopia*. Bot. Mag. (Tokyo) 82: 287–293.
- . 1970a. Structure and development of seeds in *Putranjiva roxburghii* Wall. J. Indian Bot. Soc. 49: 99–105.
- . 1970b. Structure and development of seeds in Euphorbiaceae: *Jatropha* species. Beitr. Biol. Pflanzen. 47: 79–90.
- . 1972. Structure and development of seed in *Phyllanthus niruri* L. J. Indian Bot. Soc. 51: 73–77.
- & S. CHOPRA. 1970. Structure and development of seeds in *Croton bonplandianum* Baill. Phytomorphology 20: 83–87.
- & J. L. JAIN. 1965. Development of female gametophyte in *Euphorbia pilosa* L. Curr. Sci. 34: 611–612.
- & A. PAL. 1968. Structure and development of seeds in Euphorbiaceae: *Dalechampia roezeliana* Muell.-Arg. Pp. 65–74 in Techn. Commun. Natl. Bot. Gard.
- SINGH, V. & A. SINGH. 1975. Placentation in Euphorbiaceae. Ann. Bot. 39: 1137–1140.
- STEBBINS, G. L. 1974. Flowering Plants: Evolution above the Species Level. Belknap Press of Harvard Univ. Press, Cambridge, Massachusetts.
- SWAMY, B. G. L. & P. M. GANAPATHY. 1957. A new type of endosperm haustorium in *Nothapodytes foetida*. Phytomorphology 7: 331–336.
- TAKHTAJAN, A. 1980. Outline of the classification of flowering plants (Magnoliophyta). Bot. Rev. 46: 225–359.
- . 1983. The systematic arrangement of dicotyledonous families. Pp. 180–201 in C. R. Metcalfe & L. Chalk (editors), 2nd ed., Vol. 2. Clarendon Press, Oxford.
- . 1987. Systema Magnoliophytorum. Institutum Botanicum Nominis V.L. Komarovii, Academia Scientiarum URSS, Leningrad.
- TATEISHI, S. 1927. On the development of the embryo sac and fertilization of *Acalypha australis* L. Bot. Mag., Tokyo 41: 477–485.
- THATHACHAR, T. 1952. Morphological studies in the Euphorbiaceae: I—*Acalypha lanceolata* Willd. Phytomorphology 2: 197–201.
- . 1953a. Morphological studies in the Euphorbiaceae. II. *Mallotus philippensis* M. Arg. Proc. Natl. Inst. Sci. India, B. 19: 469–474.
- . 1953b. Morphological studies in the Euphorbiaceae. J. Mysore Univ. 13B: 363–388.
- THORNE, R. F. 1983. Proposed new realignments in the angiosperms. Nordic J. Bot. 3: 85–117.
- VENKATESWARLU, J. & P. N. RAO. 1963. Endosperm in Euphorbiaceae and occurrence of endosperm haustoria in two species of *Croton* Linn. Curr. Sci. 32: 514–516.
- , ——— & D. S. RAO. 1973. Occurrence of stylar obturator in two Euphorbiaceae. Curr. Sci. 43: 128–129.
- VENTURA, M. 1934. Sulla poliembrionia di *Mallotus japonicus* Muell. Arg. Ann. Bot. (Roma) 20: 568–578.
- WAGENITZ, G. 1975. Flower reduction as central problem of angiosperm taxonomy. Bot. Jahrb. 96: 448–470.
- WEBSTER, G. L. 1967. The genera of Euphorbiaceae in the southeastern United States. J. Arnold Arbor. 48: 303–430.
- . 1975. Conspectus of a new classification of the Euphorbiaceae. Taxon 24: 593–601.
- . 1987a. The saga of the spurges: A review of classification and relationships in the Euphorbiales. Bot. J. Linn. Soc. 94: 3–46.
- . 1987b. Evolution and distributional history of the Euphorbiaceae. P. 309 in XIV Int. Bot. Congress, Berlin. [Abstract.]
- & E. A. RUPERT. 1973. Phylogenetic significance of pollen nuclear number in the Euphorbiaceae. Evolution 27: 524–531.
- , ——— & D. KOUTNIK. 1982. Systematic significance of pollen nuclear number in Euphorbiaceae, tribe Euphorbieae. Amer. J. Bot. 69: 407–415.
- & B. D. WEBSTER. 1972. The morphology and relationships of *Dalechampia scandens* (Euphorbiaceae). Amer. J. Bot. 59: 573–586.
- WIEHR, E. 1930. Beiträge zur Kenntnis der Anatomie der wichtigsten Euphorbiaceen Samen unter besonderer Berücksichtigung ihrer Erkennungsmerkmale in Futtermitteln. Landw. Versuchsstationen 110: 313–398.
- WIGER, J. 1935. Embryological Studies on the Families Buxaceae, Meliaceae, Simarubaceae and Burseraceae. Ph.D. Thesis. Univ. of Lund, Lund.
- WUNDERLICH, R. 1967. Some remarks on the taxonomic significance of the seed coat. Phytomorphology 17: 301–311.

LEGUMINLIKE PROTEINS AND THE SYSTEMATICS OF THE EUPHORBIACEAE¹

Uwe Jensen,² Ina Vogel-Bauer,²
and Marei Nitschke²

ABSTRACT

Serological methods were used to elucidate legumin similarities, i.e., similarities between the major seed storage proteins present in the Euphorbiaceae and related families. The results support the separation of two rather than five subfamilies in the Euphorbiaceae. Extrafamilial close relationships were detected with taxa from Violiflorae, Malviflorae (both subclass Dilleniidae), and Rutiflorae (subclass Rosidae). Therefore, maintaining these two subclasses as natural categories is not supported by the data reported here.

The Euphorbiaceae, comprised of approximately 8000 species and 300 genera, include a wide range of morphological diversity, such as broad-leaved trees from the tropical rainforest, succulents from arid ecosystems, annual weeds, and even a *Salvinia*-like water plant (*Phyllanthus fluitans* Benth.). Because of this great diversity, which includes substantial family characteristics (latex, number of seeds/carpels, basic chromosome numbers), the taxonomic position of the family continues to be in dispute (Webster, 1987). Are the Euphorbiaceae phyletically and taxonomically connected to orders included in the Dilleniidae (e.g., Dahlgren, 1983; Frohne & Jensen, 1992), the Rosidae (e.g., Ehrendorfer, 1991) or both (e.g., Baillon, 1858; Pax, 1924)? Although most authors consider the Euphorbiaceae to have arisen monophyletically, the strongly indicated separation into two (Dahlgren, 1980) or five (Webster, 1975, and 1994) subfamilies gives rise to speculation of di- or polyphyletic origin (Schultes, 1987).

The classification dilemma illustrates the need for additional taxonomic and phylogenetic criteria to improve our understanding of the systematics of this family. Molecular data obtained from the investigation of nucleic acids and proteins conducted for other taxonomic groups have proven to be valuable for such taxonomic research. In this investigation we utilized comparative data from legumin molecules, proteins that have been successfully included in the systematic research for similar projects (e.g., Jensen & Greven, 1984; Bergner & Jensen, 1989).

Leguminlike proteins are the major seed proteins in gymnosperms (Jensen & Berthold, 1989, for *Ginkgo*, and unpublished results for the conifers and Gnetatae) and angiosperms (Jensen & Büttner, 1981; Jensen & Grumpe, 1983; Jensen & Greven, 1984; Jensen, 1984; Bergner & Jensen, 1989; Fischer & Jensen, 1992). These proteins have proven to be important characters for the elucidation of phylogenetic relationships in similar investigations. Thus, a priori statements have been based on the omnipresence of leguminlike proteins in seed plants plus their relatively high variability in evolution. A posteriori statements have been supported by the evidence seed proteins, including legumin, have provided in a variety of systematic studies (Cristofolini, 1980; Fairbrothers et al., 1975; Fairbrothers, 1977, 1983; Jensen & Fairbrothers, 1983).

METHODS AND MATERIALS

Immunogenic legumins were isolated from the seeds of *Phyllanthus calycinus* Labill. (Phyllanthoideae), *Ricinus communis* L. (Acalyphoideae), *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg., *Reutealis trisperma* (Blanco) A. Shaw (both Crotonoideae), and *Hura crepitans* L. (Euphorbioideae). Sources of the seeds are given in the Appendix. The purified proteins were injected into rabbits to elicit specific antisera ("reference systems"). These antisera and the antigens from about 30 Euphorbiaceae species and 100 non-Euphorbiaceae species were used in the subsequent two-

¹ We gratefully acknowledge the assistance of Jörg Herbst for the statistic analysis, and the revision of the manuscript by D. E. Fairbrothers. This investigation was partially funded by grants from the Deutsche Forschungsgemeinschaft.

² Lehrstuhl für Pflanzenökologie und Systematik, Universität Bayreuth, D-95440 Bayreuth, Germany.

dimensional immunodiffusion experiments on agar plates. The similarities and dissimilarities between the legumins from the different taxa were revealed qualitatively by spur-analysis of the precipitates (Lester et al., 1983). For details, see Vogel (1986).

Such data of relative similarity have proven to be relevant for systematic interpretation, because they separate qualitative units; quantitative measurements for the characterization of systematic similarities have proven useless when investigating multicharacter systems. The multicharacter systems used are represented by the reference antisera; each reference antiserum contains many kinds of antibodies specifically raised against the different determinants of the legumin molecule of one species. These different kinds of antibodies included in one monospecific antiserum are the "reference characters" in our systematic research.

RESULTS

INTRAFAMILIAR LEGUMIN AFFINITIES

For the five reference systems, i.e., *Phyllanthus calycinus*, *Ricinus communis*, *Hevea brasiliensis*, *Reutealis trisperma*, and *Hura crepitans*, the data from the mutual comparison of precipitates from all pairs of taxa used (i.e., A spurs B, B spurs A, A spurs B and B spurs A, A is identical with B) are listed in data matrices (Table 1a-e). Taxa in the data matrices are ranked according to the serological similarity in relation to the reference system.

For the systematic evaluation of the taxa included in this investigation, two criteria are especially important. (i) The relative position of serological similarity in relation to the reference taxon. This position is more or less given by the ranking in Table 1 and also by the simplified ranking groups shown in Figure 1. (ii) Identical serological reactivities of two cross-reacting taxa are important if their serological reactivity is close to the reactivity of the reference taxon. For example, the serological identity between *Andrachne colchica* Fisch. et Mey. ex Boiss. and *Securinega durissima* Gmel. is significant, since the high correspondence of both antigens to the reference system of *Phyllanthus calycinus* includes a large portion of their legumin molecule (Table 1a). In contrast, the identical reaction of *Jatropha curcas* L. and *Aleurites montana* (Lour.) Wilson cannot be used to postulate identical or highly similar legumins because their correspondence to the reference system only represents a very small part of their potential antigenic legumin reactivity (Table 1a).

The data are presented on the basis of a dis-

crimination of the five subfamilies: Phyllanthoideae, Oldfieldioideae, Acalyphoideae, Crotonoideae, and Euphorbioideae, following Webster's classification (1975, 1994). No reference system from the Oldfieldioideae has been employed in this research.

Phyllanthoideae. Using *Phyllanthus calycinus* as a reference system (Table 1a and Fig. 1a), the other Phyllanthoideae genera *Reverchonia*, *Breyenia*, *Andrachne*, *Securinega*, and—somewhat separated—*Margaritaria*, proved to be the most similar taxa (i.e., had the highest similarity indices). *Antidesma bunius* Spreng., another member of the Phyllanthoideae, is distantly connected. The reactions of all Crotonoideae and Acalyphoideae taxa did not exceed the outgroup reactivities; only for the Euphorbioideae *Homalanthus*, *Sapium*, and *Hura*, have relationships indicating affinities been observed.

Acalyphoideae. *Chrozophora* and *Mercurialis*, members of the subfamily Acalyphoideae, revealed the expected high similarity indices with the *Ricinus communis* reference system (Table 1b, and Fig. 1b). The similarity indices between *Ricinus* and the other two members of the Acalyphoideae, i.e., *Dalechampia* and especially *Mallotus*, are definitely lower. *Joannesia* (Crotonoideae) is high in similarity ranking and represents significant affinities between Crotonoideae and Acalyphoideae. *Hevea* (Crotonoideae), *Hura*, and *Homalanthus* (both Euphorbioideae) are serologically similar to each other. All Phyllanthoideae and Oldfieldioideae react in the range of Euphorbiaceae outgroups.

Crotonoideae. Two taxa revealed the highest similarity indices with the *Hevea brasiliensis* (Crotonoideae) reference system (Table 1c and Fig. 1c): *Manihot* (Crotonoideae) and *Ricinus* (Acalyphoideae). However, the serological similarities between both taxa and *Hevea* include different parts of the spectrum of serological reactivity. Next to these two genera, *Croton* (Crotonoideae), as well as *Homalanthus* and *Hura* (both Euphorbioideae), have more serological legumin characters in common with *Hevea* than the other Euphorbiaceae genera tested. The Phyllanthoideae and Oldfieldioideae genera produce low reactivity. The serological reactions of *Drypetes*, *Phyllanthus*, *Andrachne*, and *Securinega* (all Phyllanthoideae) with the *Hevea* antisystem were identical; the legumin precipitation of *Antidesma*, another member of the Phyllanthoideae, "spurs" the legumin precipitation of the four genera indicating a somewhat higher degree of similarity with *Hevea*.

Another reference system from the subfamily Crotonoideae, *Reutealis trisperma* (tribe Aleuri-

TABLE 1. Data matrices for the serological results from two 1-dimensional gel diffusion experiments using five reference systems, i.e., *Phyllanthus calycinus* Labill. (a), *Ricinus communis* L. (b), *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. (c), *Reutealis trisperma* (Blanco) A. Shaw (d), *Hura crepitans* L. (e). | legumin of the left species spurs the upper species; - legumin of the upper species spurs the left species; + double spurring; O identical precipitation; ● blank. All generic names are spelled out in the Appendix.

The data are shown symmetrically. Boxes indicate taxa groups with identical or similar cross-reactivity. The interrupted line marks the maximum reactivity of the outgroups.

Right column: Similarity indices calculated according to the formula

$$S_i = \frac{S_c}{S_R} \times 100 (\%)$$

$S = \Sigma | + \frac{1}{2} \Sigma + + \frac{1}{2} \Sigma O$ for each species, using the row data, where R = reference taxon and C = cross-reacting taxon.

		a																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	S_i
<i>Phyllanthus calycinus</i>	1	●																								100
<i>Reverchonia arenaria</i>	2	-	●	○																						93
<i>Breynia fruticosa</i>	3	-	○	●	○	○																				(81)
<i>Andrachne colchica</i>	4	-	-	○	●	○																				87
<i>Securinega durissima</i>	5	-	-	○	○	●																				87
<i>H. populifolius</i>	6	-	-	-	-	-	●	○	○	+																87
<i>Sapium sebiferum</i>	7	-	-	-	-	-	○	●	○	+																72
<i>Hura crepitans</i>	8	-	-	-	-	-	○	○	●	+																72
<i>Margaritaria scandens</i>	9	-	-	-	-	-	+	+	+	●	+															72
<i>Omphalea trichotoma</i>	10	-	-	-	-	-	-	-	-	+	●	+	+	+	+		+									50
<i>Adelia ricinella</i>	11	-	-	-	-	-	-	-	-	+	●	+	+	+	+		+									52
<i>Ricinus communis</i>	12	-	-	-	-	-	-	-	-	+	+	+	●	+	+	○	+	+								50
<i>Manihot glaziovii</i>	13	-	-	-	-	-	-	-	-	+	+	+	+	●	+	○		+								48
<i>Hevea brasiliensis</i>	14	-	-	-	-	-	-	-	-	+	+	+	+	+	●	○	+	+								50
<i>Mercurialis annua</i>	15	-	-	-	-	-	-	-	-	+	-	-	○	○	○	●	+									48
<i>Antidesma bunius</i>	16	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	●	+								46
<i>Chrozophora tinctoria</i>	17	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+	+	●	○	○	○				46
<i>S. fimbriicalyx</i>	18	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	○	○	○	○	○				35
<i>Joannesia princeps</i>	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	○	○	○			22
<i>Mallotus tenuifolius</i>	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	○	○	○			22
<i>Euphorbia characias</i>	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	○	○	○	○		17
<i>Aleurites montana</i>	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	○	○	○	○	15
<i>Croton tiglium</i>	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	○	○	○	○	11
<i>Jatropha curcas</i>	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	○	○	○	9
																										4

TABLE 1. Continued.

		c																															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	S _i	
<i>Hevea brasiliensis</i>	1	●																													100		
<i>Manihot esculenta</i>	2	-	●	+																											95		
<i>Ricinus communis</i>	3	-	+	●					+				+																		91		
<i>H. populifolius</i>	4	-	-	-	●	+	+																								86		
<i>Hura crepitans</i>	5	-	-	-	+	●	+						+																		84		
<i>Croton tiglium</i>	6	-	-	-	+	+	●		+		+												+								81		
<i>Joannesia princeps</i>	7	-	-	-	-	-	-	●		+								+													76		
<i>Chrozophora tinctoria</i>	8	-	-	+	-	-	+	-	●	+		-			+																	72	
<i>Sapium sebiferum</i>	9	-	-	-	-	-	-	+	+	●	+	+				+																71	
<i>Reutealis trisperma</i>	10	-	-	-	-	-	+	-	-	+	●	+	+			+																67	
<i>Euphorbia lathyris</i>	11	-	-	-	-	-	-	-		+	+	●	+	+					+				+									66	
<i>Excoecaria bicolor</i>	12	-	-	+	-	-	-	-	-	-	+	+	●			-		+														62	
<i>Jatropha curcas</i>	13	-	-	-	-	+	-	-	-	-	-	+	-	●		+		+														59	
<i>Dalechampia scandens</i>	14	-	-	-	-	-	-	-	+	-	-	-	-	-	●							+										55	
<i>Mercurialis perennis</i>	15	-	-	-	-	-	-	-	-	+	+	-	-	-	-	●		-														52	
<i>Aleuritis montana</i>	16	-	-	-	-	-	-	-	-	-	-	-		+	-	-	●		+													52	
<i>Mallotus philippinensis</i>	17	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	●		+													50	
<i>Putranjiva roxburghii</i>	18	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	●	+	+	-										41	
<i>Vernicia montana</i>	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	●		-	+	+								36	
<i>Eremocarpus spec.</i>	20	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	●										38	
<i>Antidesma bunius</i>	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	●									34	
<i>Drypetes australasica</i>	22	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	●	○	○	○					28	
<i>Phyllanthus grandifolius</i>	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	○	●	○	○					24	
<i>Andrachne colchica</i>	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	●	○		○			21	
<i>Securinega suffrutic.</i>	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	●		○			19	
<i>Baccaurea tetrandra</i>	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	●			14	
<i>Glochidion glaucum</i>	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	-	●			10	
<i>Bridelia tomentosa</i>	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	-	○	●			9
<i>Micrantheum ericoides</i>	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	●			7
<i>Petalostigma quadriloc.</i>	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	0	

TABLE 1. Continued.

		e																															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	S _i	
<i>Hura crepitans</i>	1	●																														100	
<i>Ricinus communis</i>	2	-	●						+																							95	
<i>Excoecaria bicolor</i>	3	-	-	●	○																											91	
<i>H. populifolius</i>	4	-	-	○	●	○																										90	
<i>Sapium sebiferum</i>	5	-	-	-	○	●																										88	
<i>Joannesia princeps</i>	6	-	-	-	-	-	●																									83	
<i>Chrozophora tinctoria</i>	7	-	-	-	-	-	●			-	-		-																			69	
<i>Manihot esculenta</i>	8	-	+	-	-	-	-	●																								78	
<i>Jatropha curcas</i>	9	-	-	-	-	-	-	-	●																							76	
<i>Hevea brasiliensis</i>	10	-	-	-	-	-	-	-	-	●			+	+																		69	
<i>Mercurialis perennis</i>	11	-	-	-	-	-	-	-	-	-	●			-																		62	
<i>Reutealis trisperma</i>	12	-	-	-	-	-	-		-	-	-	-	●																			66	
<i>Croton tiglium</i>	13	-	-	-	-	-	-	-	-	-	+	-	●		+																	59	
<i>Euphorbia lathyris</i>	14	-	-	-	-	-	-	-	-	-	+		-	-	●																		60
<i>Aleurites montana</i>	15	-	-	-	-	-	-	-	-	-	-	-	-	+	-	●																	53
<i>Vernicia montana</i>	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●		+				+					+				43	
<i>Dalechampia scandens</i>	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●															45
<i>Eremocarpus setigerus</i>	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	●				+										41
<i>Mallotus philippinensis</i>	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●													38
<i>P. quadriloculare</i>	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●		-									31
<i>Micranthemum ericoides</i>	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●										31
<i>Securinega suffruticosa</i>	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-		-	●	○								33
<i>Phyllanthus grandifolius</i>	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	●	○	○						22
<i>Andrachne colchica</i>	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	●	○					21
<i>Bridelia tomentosa</i>	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	○	●					21
<i>Antidesma bunius</i>	26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
<i>Drypetes australasica</i>	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	○	○	○		9
<i>Putranjiva roxburghii</i>	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	●	○	+		9
<i>Baccaurea tetrandra</i>	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	○	●	○		9
<i>Glochidion glaucum</i>	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	○	+	○	●		7
																																	0

tideae), has been tested (Table 1d and Fig. 1d). Clearly the two other members of the tribe Aleuritideae, i.e., *Vernicia* and *Aleurites*, have proved to be the most similar taxa. The ranking of the other Crotonoideae genera was graded in the following decreasing order: *Jatropha*–*Joannesia*–*Croton*–*Hura*–*Manihot*–*Eremocarpus*. Again, the Phyllanthoideae showed the least legumin affinities; however, additional taxa were included in this group of low affinities: *Eremocarpus* (Crotonoideae) and especially taxa with strong serological connections with the *Hevea* reference system, i.e., *Hevea* (Crotonoideae), *Sapium*, *Homalanthus* (Euphorbioideae), and *Ricinus* (Acalyphoideae).

Euphorbioideae. The highest similarity indices with the *Hura crepitans* (Euphorbioideae) antisystem (Table 1e and Fig. 1e) have been observed for *Ricinus communis*, which is placed in the Acalyphoideae. The serologically identical genera *Excoecaria*, *Homalanthus*, and *Sapium* (all Euphorbioideae) follow. *Euphorbia* reveals affinities that are lower than most members of the Acalyphoideae and Crotonoideae. The similarity indices of the Phyllanthoideae and Oldfieldioideae are again the lowest and do not exceed the outgroup reactivities.

For a graphic representation of the data patterns and relationships between taxa, ordination techniques were preferred over cluster analyses. Cluster analyses like UPGMA or similar hierarchical methods lead to one-dimensional representations accompanied by a considerable loss of information compared to two- or three-dimensional ordinations. Also, the results of the reference experiments do not deliver a complete matrix of pairwise similarities, but they can be treated as five variables measuring identities in relation to the reference taxa. Such data are better analyzed by dimension reduction or ordination techniques than by cluster analyses. The resulting graphs simultaneously show similarities between all taxa and allow visual inspection of their relationships.

For the five reference taxa, the similarity values can be presented two-dimensionally with little deviation from the experimentally obtained data (Fig. 2). This graph clearly demonstrates close serological similarities between *Ricinus*, *Hura*, and *Hevea*, but a distant position for *Phyllanthus* is revealed.

Two scaling procedures, i.e., Principal Component Analysis (PCA) and Nonlinear Multidimensional Scaling (NMDS) (Rohlf, 1989) have been used to calculate multidimensional graphs for the five reference taxa plus the 13 genera that have been included in all five reference experiments (Fig. 3a, b). Since relative positions for the taxa included

in both graphs (based on different, nonrelated statistical procedures) principally are identical, they adequately represent the relationships between the taxa. For the five reference taxa the relative positions are almost the same as shown in Figure 2.

With the additional taxa, the Phyllanthoideae are clearly separated from the Acalyphoideae + Crotonoideae + Euphorbioideae. Within the Phyllanthoideae, *Antidesma* is only distantly connected. This is also the case for *Mallotus* within the Acalyphoideae. When compared with other members of the Crotonoideae (e.g., *Hevea*), the Aleuritideae (*Aleurites*, *Reutealis*) are distantly located. *Euphorbia* is separated from the Hippomaneae (*Homalanthus*, *Sapium*) within the Euphorbioideae.

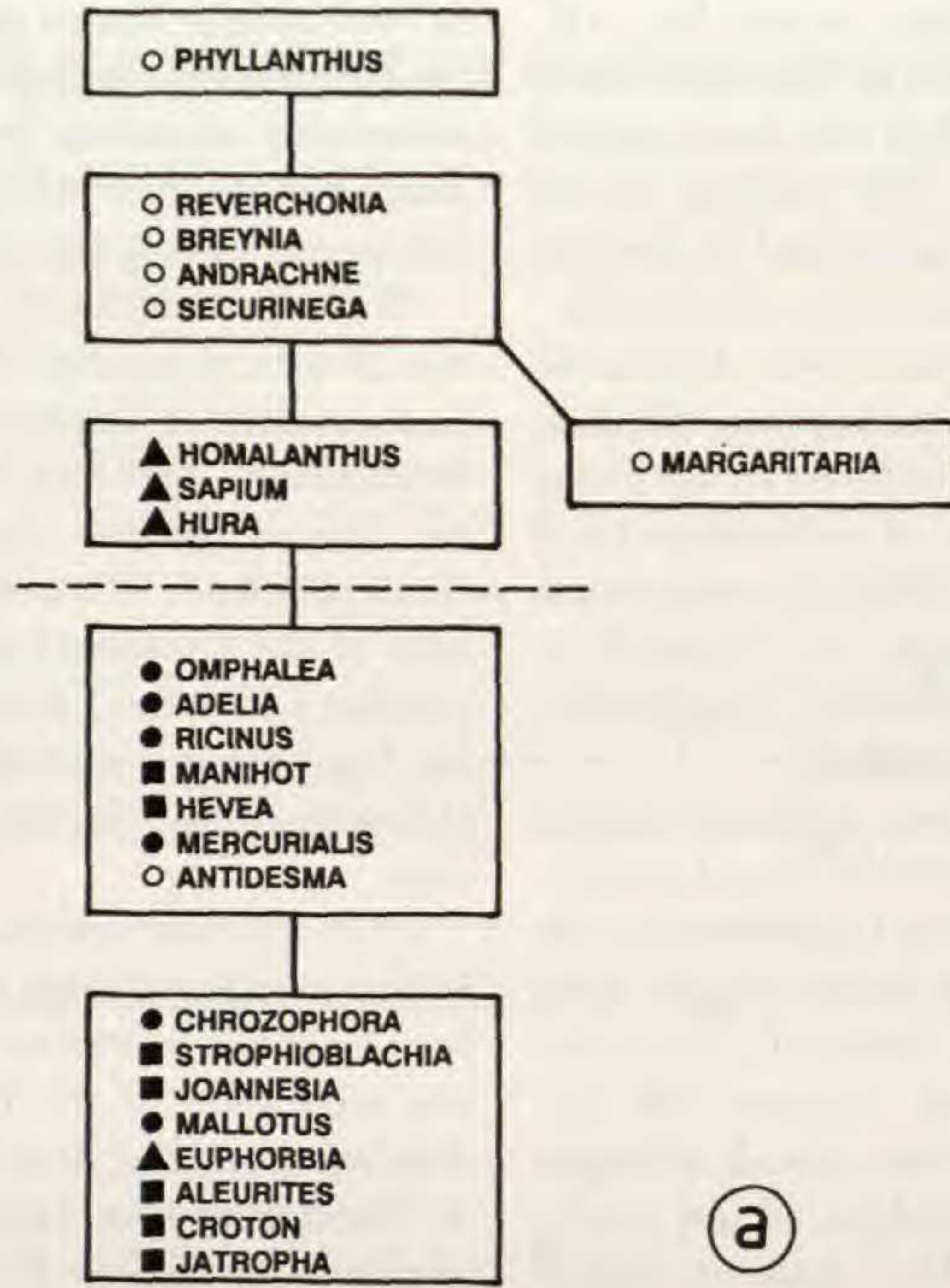
PCA has also been used for an ordination of a higher number of taxa, i.e., those that are included in at least four reference systems (Fig. 4). Although the separation of the Phyllanthoideae + Oldfieldioideae from the Acalyphoideae + Crotonoideae + Euphorbioideae becomes less distinct, a high similarity to Figure 3 can be observed. The resulting pattern again demonstrates the missing serological discrimination capacity between *Ricinus* (Acalyphoideae), *Hura* (Euphorbioideae), and *Hevea* (Crotonoideae) reference systems. On the contrary, the taxa connected to *Hevea* and *Reutealis* (both Crotonoideae) are clearly separated.

EXTRAFAMILIAR CONNECTIONS

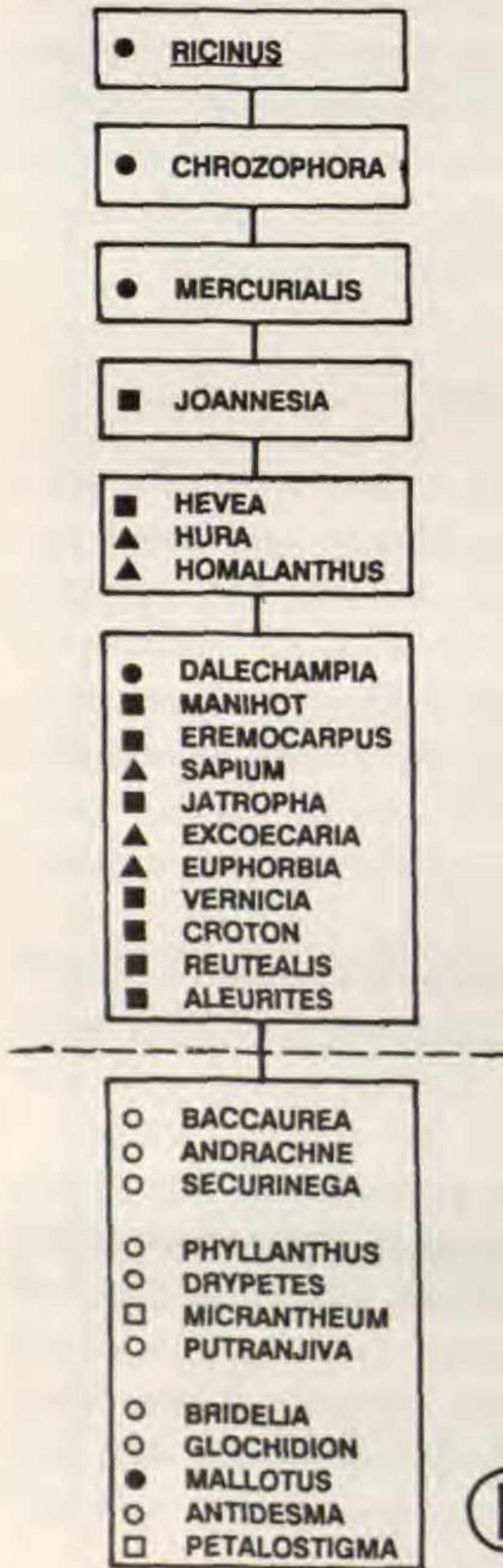
For the elucidation of the major systematic connections between Euphorbiaceae and other angiospermous families, we investigated approximately 100 species from a large number of dicotyledonous plant families. These taxa were tested for the serological reaction of their leguminlike proteins with the antiserum against *Ricinus communis*. One to three species were used to represent each family tested.

Families with very low legumin similarity indices (= no or very weak serological reactions) were Asteraceae, Lauraceae, Pittosporaceae, and Ranunculaceae; families with low legumin similarity indices (= weak serological reactions) are shown in Table 2. Only the species of Table 3 revealed high legumin similarity indices, exceeding those of many Euphorbiaceae species (ranking graduated according to their relative serological reactivity with the *Ricinus communis* antisystem, with the Passifloraceae species as the most similar species; see tab. 9 in Vogel, 1986).

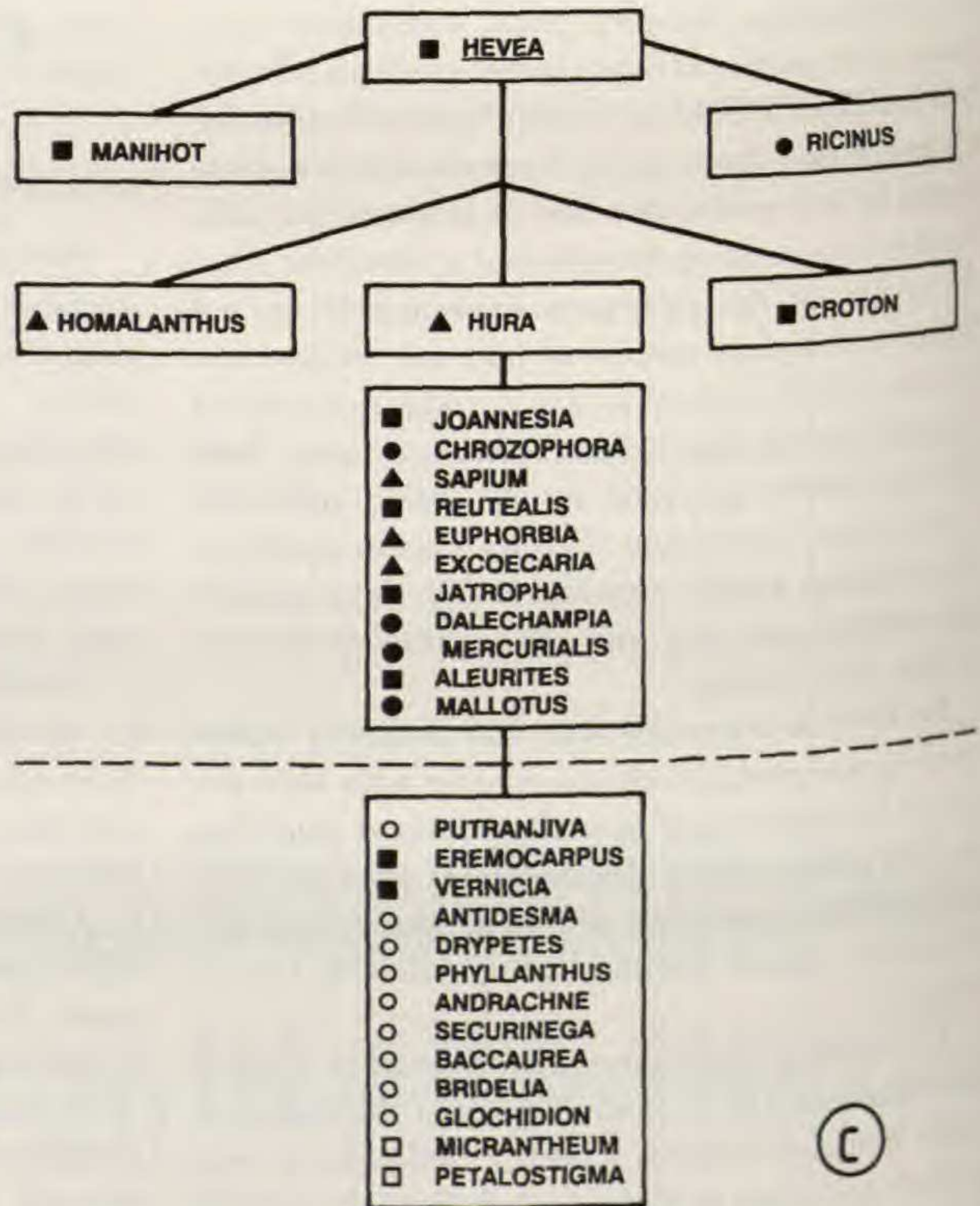
All taxa of high legumin similarity (Table 3) belong to the superorders Malviflorae, Rutiflorae, and Violiflorae. They are of interest in attempting



(a)

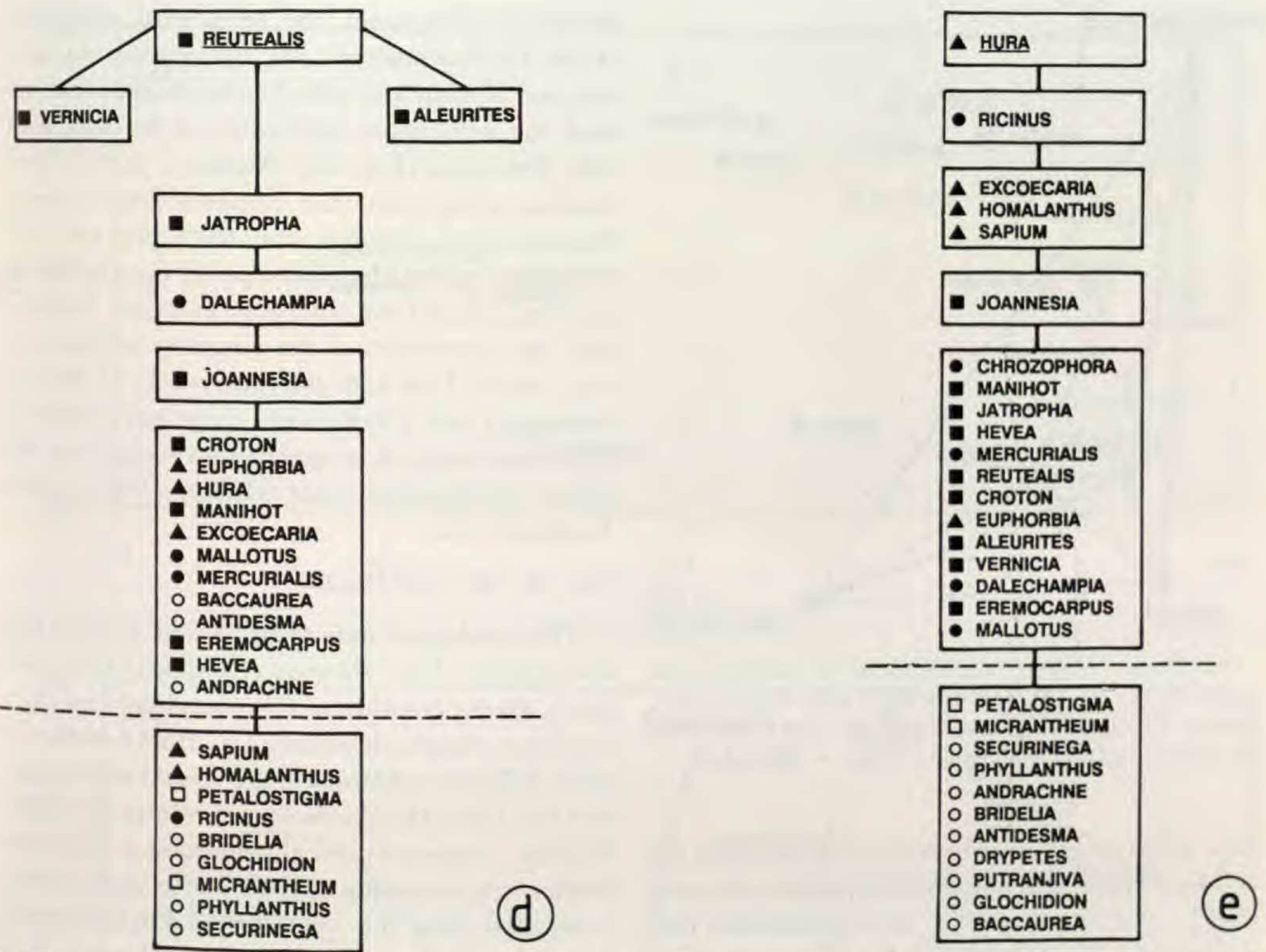


(b)



(c)

FIGURE 1. Simplified interpretation of the relative serological similarities in relation to each reference system. i.e., *Phyllanthus calycinus* (a), *Ricinus communis* (b), *Hevea brasiliensis* (c), *Reutealis trisperma* (d), *Hura crepitans*



(e). The interrupted line marks the maximum reactivity of the outgroups. Only generic names are used because of supposedly insignificant or undetectable serological infrageneric differences. ○ Phyllanthoideae; □ Oldfieldioideae; ● Acalyphoideae; ■ Crotonoideae; ▲ Euphorbioideae.

to interpret the relationships of the Euphorbiaceae to the other dicotyledons.

Species from the 11 families listed in Table 3 and another 18 families also belonging to the three superorders, plus one species each from the non-related Magnoliaceae, Scrophulariaceae, and Fabaceae were compared in a two-dimensional gel diffusion experiment using *Ricinus communis* as the reference antisystem. For a similar experiment using the *Phyllanthus calycinus* reference antisystem, only species from 11 top reacting families were included (except the Tropaeolaceae, since the protein material failed to react). The data matrix is given in Table 4. For all Malviflorae, Violiflorae, and Rutiflorae, the serological similarities are included in Figure 5.

DISCUSSION

THE EUPHORBIACEAE—ONE FAMILY OR NOT?

The Phyllanthoideae (and Oldfieldioideae, which are only marginally considered in this investigation) differ in prominent characters from the other subfamilies, i.e., Acalyphoideae, Crotonoideae, and

Euphorbioideae: the biovular locule (in contrast to the uniovular locule, characteristic for the other three subfamilies), the lack of latex and laticifers (which are present in almost all Acalyphoideae), and the basic chromosome number of 12 or 13 (Webster, 1975). The testa of the seeds are never developed in the form of palisades (Corner, 1976).

Although in most modern classifications the Phyllanthoideae are proposed to constitute a subfamily of the Euphorbiaceae, Huber (1985) proposed this taxon as a separate family that should be incorporated into the Linales. Bentham (1880) connected the Phyllanthoideae with the Rhamnales, with the exception of the "Hippomaneae," which he placed within the Urticales. Pax (1924) proposed a polyphyletic origin for the Euphorbiaceae, from both the Malvales and Geraniales.

Our serological results indicate that the legumins of the Phyllanthoideae are different from those of the Acalyphoideae, Crotonoideae, and Euphorbioideae (Fig. 3). In many cases (reference systems of *Hura* (Euphorbioideae), *Ricinus* (Acalyphoideae), *Hevea* (Crotonoideae)), the members of the Phyllanthoideae do not obviously exceed the affin-

PHYLLANTHUS

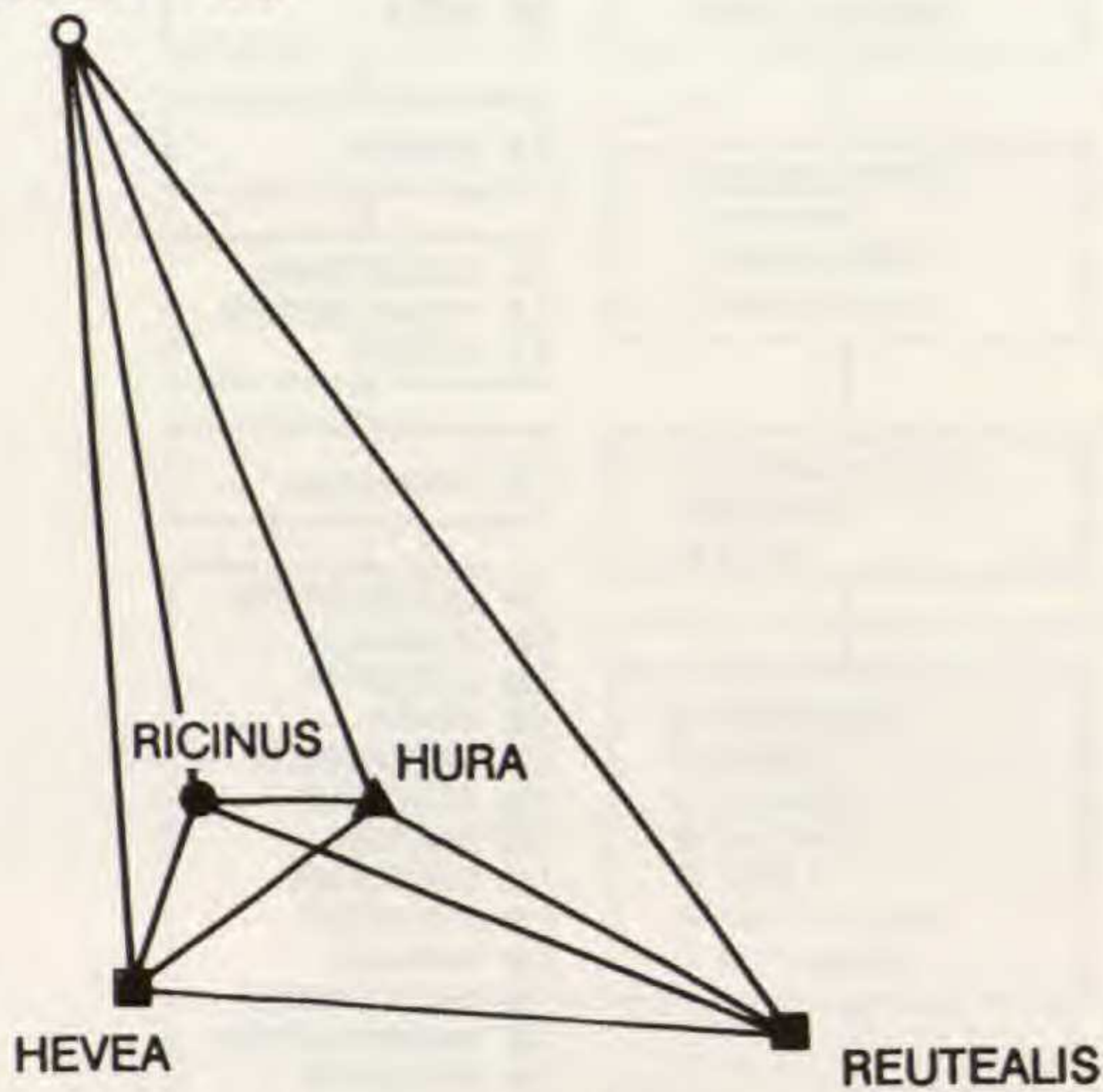


FIGURE 2. Graphic representation of serological distances (S.D.) for the five reference taxa *Phyllanthus*, *Hevea*, *Ricinus*, *Hura*, and *Reutealis*, calculated from the Similarity Indices of Table 1 (S.D. = $100 - S_i$).

ities of the outgroups. However, in some cases our results indicate that the Phyllanthoideae are more closely related to the other three subfamilies than to the outgroup families: In the *Reuteales* (Cro-

tonoideae) antisystem, the serological similarities of the Phyllanthoideae taxa *Baccaurea*, *Antidesma*, and *Andrachne* with *Reutealis* somewhat exceed the serological similarities of the outgroups with *Reutealis* (Fig. 1d). Similarly, in the *Phyllanthus* antisystem, the Euphorbioideae genera *Homalanthus*, *Sapium*, and *Hura* plus the Acalyphoideae genus *Omphalea* partly (i.e., by double spurring) exceed the outgroup reactions. Additionally, the reactivities of the outgroup antigens are very similar both with the antisystems of *Ricinus communis* and *Phyllanthus calycinus* (Table 4), and do not support proposals that suggest two different phylogenetic roots (Huber, 1985) for the Euphorbiaceae.

FIVE OR TWO SUBFAMILIES?

The serological data (Figs. 3 and 4) show that the legumins from *Ricinus communis* (Acalyphoideae), *Hevea brasiliensis* (Crotonoideae), and *Hura crepitans* (Euphorbioideae), i.e., from members of three different subfamilies, are remarkably similar. For the *Hura* (Euphorbioideae) reference system, *Ricinus communis* (Acalyphoideae) is the most similar cross-reacting species and is more closely connected than the other tested Euphorbioideae taxa from the genera *Excoecaria*, *Homalanthus*,

TABLE 2. Families, taxa of which show low legumin similarity indices with a *Ricinus communis* (Euphorbiaceae) antiserum. Classification is according to Dahlgren (1980).

Family	Order	Superorder
Araliaceae	Araliales	Araliiflorae
Berberidaceae	Ranunculales	Ranunculiflorae
Buxaceae	Buxales	Rosiflorae
Casuarinaceae	Casuarinales	Rosiflorae
Celastraceae	Celastrales	Santaliflorae
Combretaceae	Myrtales	Myrtiflorae
Daphniphyllaceae	Buxales	Rosiflorae
Fabaceae	Fabales	Fabiflorae
Fagaceae	Fagales	Rosiflorae
Gentianaceae	Gentianales	Gentianiflorae
Juglandaceae	Juglandales	Rosiflorae
Lythraceae	Myrtales	Myrtiflorae
Magnoliaceae	Magnoliales	Magnoliiflorae
Melastomataceae	Myrtales	Myrtiflorae
Myristicaceae	Annonales	Magnoliiflorae
Myrtaceae	Myrtales	Myrtiflorae
Onagraceae	Myrtales	Myrtiflorae
Papaveraceae	Papaverales	Ranunculiflorae
Primulaceae	Primulales	Primuliflorae
Proteaceae	Proteales	Proteiflorae
Rosaceae	Rosales	Rosiflorae
Saxifragaceae	Saxifragales	Rosiflorae
Scrophulariaceae	Scrophulariales	Lamiiflorae
Solanaceae	Solanales	Solaniflorae

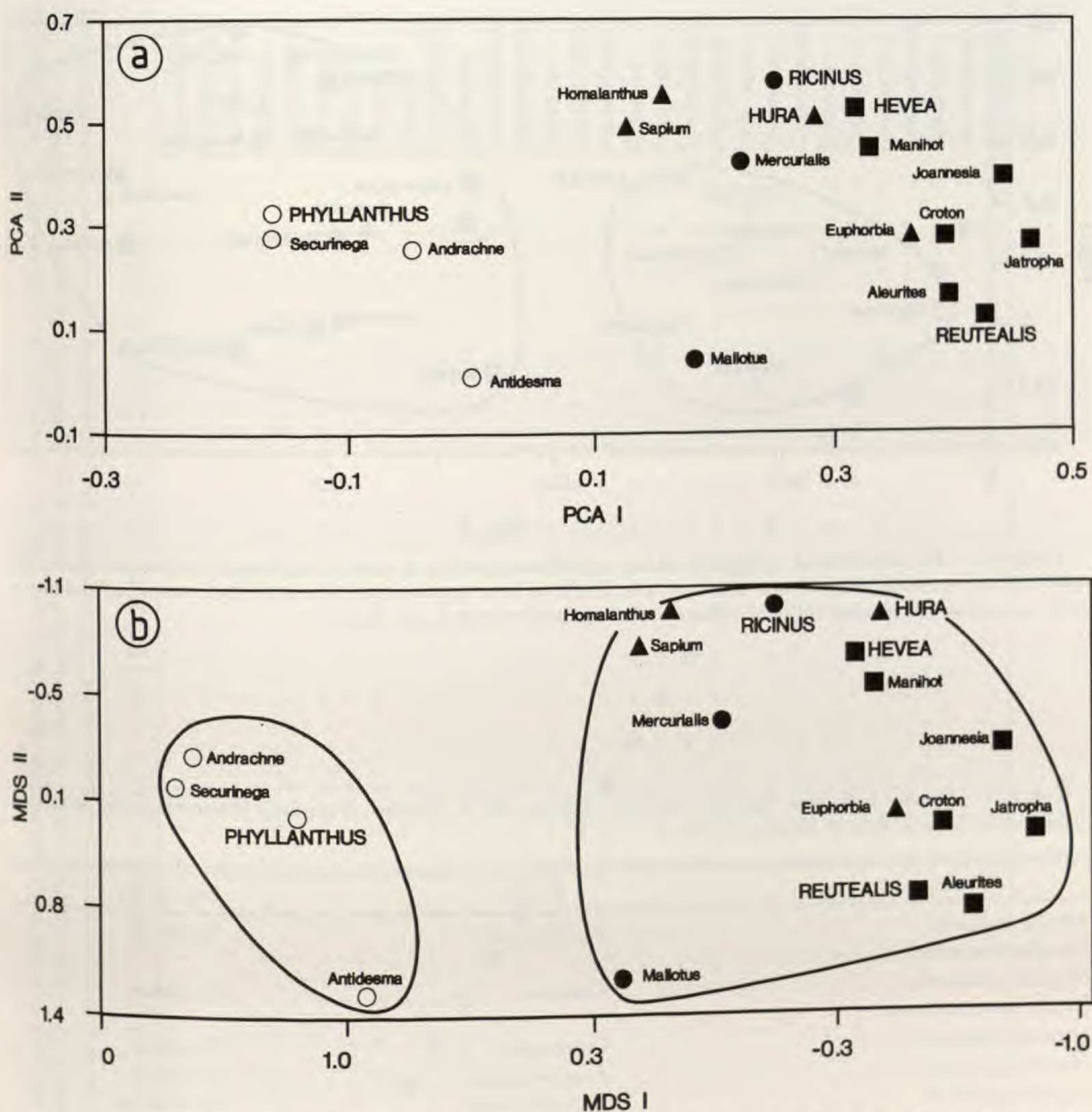


FIGURE 3. Two-dimensional ordinations of five variables equivalent to relative serological similarities between five reference taxa (capitals) and 13 nonreference taxa. For subfamily symbols see Figure 1.—a. PCA, two factors explaining 91% of variance.—b. MDS, based on pairwise average taxonomic distances (Sneath & Sokal, 1973), calculated from five variables. “Stress” = 0.095, indicating good conservation of the rank order of observed distances by the depicted interpoint distances, i.e., very good fit to observed distances.

Sapium, and *Euphorbia*. The correlation between the data of serological similarity from the three reference systems mentioned is high; the regression curve is about $y = x$ and almost identical for all correlation combinations (Vogel, 1986). This is not the case for the *Reutealis* reference system (Fig. 3), another reference system from the Crotonoideae, which indicated a considerable phylogenetic distance within the Crotonoideae in relation to the high correspondence obtained among the three subfamilies.

Thus the serological data do not support the separation of five subfamilies, but clearly indicate

the separation of the Acalyphoideae–Crotonoideae–Euphorbioideae and the Phyllanthoideae as two phylogenetic groups. This conclusion is in agreement with the taxonomic treatments of several authors who designate the two subfamilies Phyllanthoideae and Hippomaneae (e.g., Dahlgren, 1980; Ehrendorfer, 1991).

SEROLOGICAL RELATIONSHIPS WITHIN THE SUBFAMILIES

Because the Oldfieldioideae have been marginally compared, only data obtained for the Phyllan-

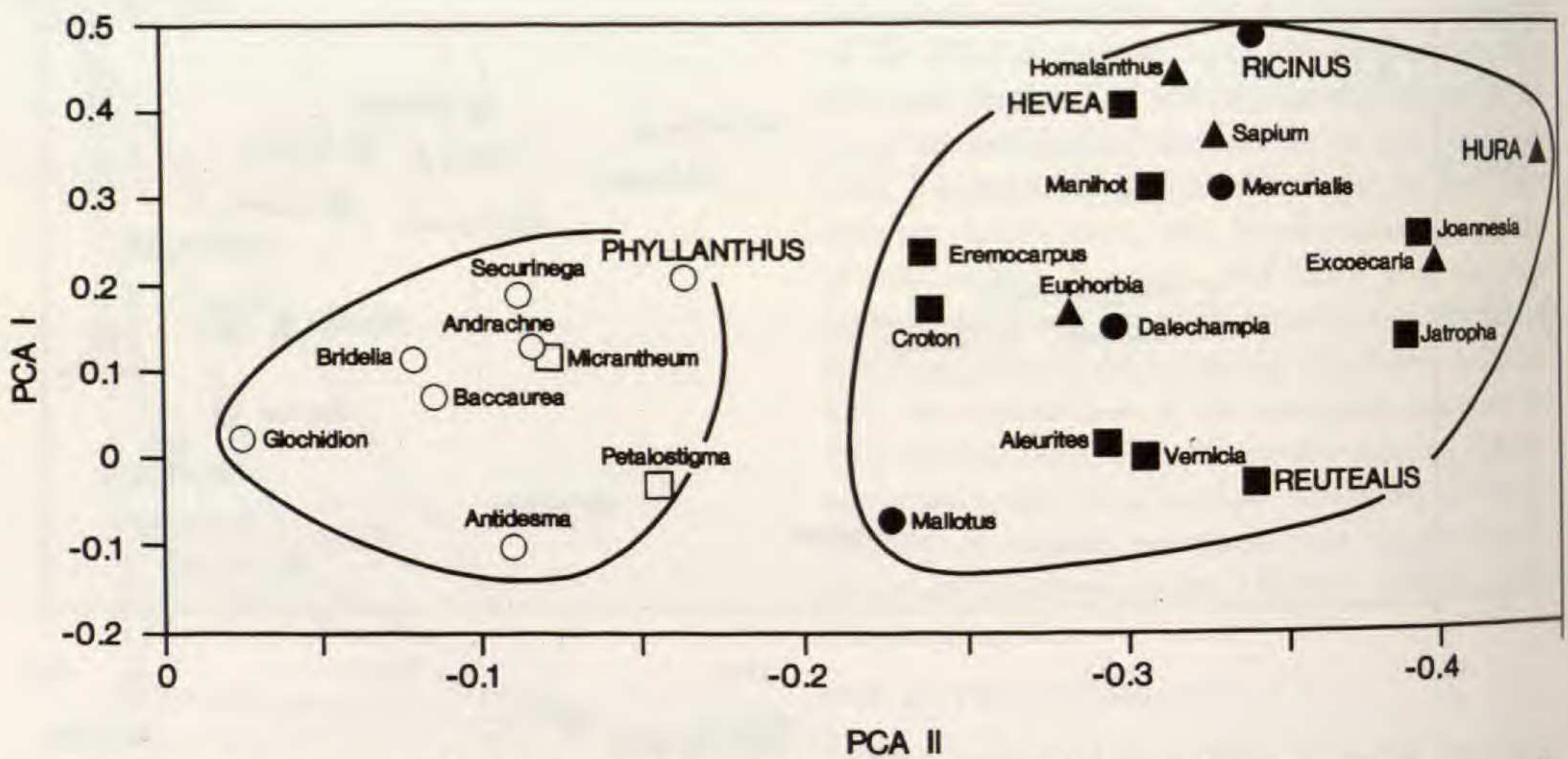


FIGURE 4. Two-dimensional ordinations of five variables equivalent to relative serological similarities between five reference taxa (capitals) and 22 nonreference taxa that have been incorporated in at least four reference experiments. PCA, two factors explaining 91% of variance. For subfamily symbols see Figure 1.

TABLE 3. Species with high legumin similarity indices with a *Ricinus communis* (Euphorbiaceae) antiserum. Classification is according to Dahlgren (1980).

Species	Family	Superorder
<i>Adenia pechuelii</i>	Passifloraceae	Violiflorae
<i>Passiflora caerulea</i>	—	
<i>Hugonia orientalis</i>	Linaceae	Rutiflorae
<i>Linum usitatissimum</i>	—	
<i>Cneorum tricoccon</i>	Cneoraceae	Rutiflorae
<i>Tropaeolum majus</i>	Tropaeolaceae	Rutiflorae
<i>Daphne gnidium</i>	Thymelaeaceae	Malviflorae
<i>Daphne mezereum</i>	—	
<i>Pimelea ligustrina</i>	—	
<i>Antiaris africana</i>	Moraceae	Malviflorae
<i>Cudrania tricuspidata</i>	—	
<i>Morus rubra</i>	—	
<i>Celtis occidentalis</i>	Ulmaceae	Malviflorae
<i>Ulmus parvifolia</i>	—	
<i>Zelkova carpinifolia</i>	—	
<i>Dictamnus albus</i>	Rutaceae	Rutiflorae
<i>Phellodendron japonicum</i>	—	
<i>Ruta graveolens</i>	—	
<i>Zanthoxylum simulans</i>	—	
<i>Casearia gladiiformis</i>	Flacourtiaceae	Violiflorae
<i>Flacourtia inermis</i>	—	
<i>Idesia polycarpa</i>	—	
<i>Laportea moroides</i>	Urticaceae	Malviflorae
<i>Parietaria officinalis</i>	—	
<i>Urtica dioica</i>	—	
<i>Anacardium occidentale</i>	Anacardiaceae	Rutiflorae
<i>Toxicodendron verniciflua</i>	—	
<i>Spondias mombin</i>	—	

TABLE 4. Data matrix for the serological results from two-dimensional gel diffusion experiments, using three outgroup taxa and the reference systems *Ricinus communis* (a) and *Phyllanthus calycinus* (b). For further explanation see Table 1. The family ending "aceae" has been removed in some cases to allow for alignment of the table.

		a																															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
<i>Ricinus communis</i>	1	●																													Euphorbiaceae		
<i>Passiflora caerulea</i>	2	-	●					+	+																						Passifloraceae		
<i>Linum usitatissimum</i>	3	-	-	●								+																			Linaceae		
<i>Cneorum tricoccum</i>	4	-	-	-	●	+									+																Cneoraceae		
<i>Tropaeolum majus</i>	5	-	-	-	+	●	+	+																							Tropaeolaceae		
<i>Daphne mezereum</i>	6	-	-	-	-	+	●		+						+																Thymelae.		
<i>Morus rubra</i>	7	-	+	-	-	+	-	●		+					+		+			+	+										Moraceae		
<i>Celtis occidentalis</i>	8	-	+	-	-	-	+	-	●	+	+		+	+	+				+			+									Ulmaceae		
<i>Dictamnus albus</i>	9	-	-	-	-	-	-	+	+	●	+	+		+																	Rutaceae		
<i>Flacourtia inermis</i>	10	-	-	-	-	-	-	-	+	+	●	+		+				+													Flacourtiaceae		
<i>Urtica dioica</i>	11	-	-	+	-	-	-	-	-	+	+	●		+	+	+			+		+										Urticaceae		
<i>A. occidentale</i>	12	-	-	-	-	-	-	-	+	-	-	-	●	+																	Anacardiaceae		
<i>Magnolia tripetala</i>	13	-	-	-	-	-	-	-	+	+	+	+	+	●	+	+		+		+		+						+			Magnoliaceae		
<i>Viola odorata</i>	14	-	-	-	-	-	+	+	+	-	-	+	-	+	●	+		+	+	+	+		+								Violaceae		
<i>Hippophae rhamnoides</i>	15	-	-	-	+	-	-	-	-	-	+	-	+	+	●	+		+		+		+									Elaeagnaceae		
<i>Sapindus saponaria</i>	16	-	-	-	-	-	-	+	-	-	-	-	-	-	+	●	+	+													Sapindaceae		
<i>Cedrela odorata</i>	17	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	●	+	+	+		+	+	+							Meliaceae		
<i>Digitalis purpurea</i>	18	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	●	+	+		+	+				+			Scrophulari.		
<i>Terminalia catappa</i>	19	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	+	+	●	+		+								Combretaceae		
<i>D. calycinum</i>	20	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	-	+	+	+	●		+	+	+	+	+				Daphniphyll.		
<i>Lythrum salicaria</i>	21	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	●		+							Lythraceae		
<i>Erodium botrys</i>	22	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	-	●	+	+		+				Geraniaceae		
<i>Cucurbita maxima</i>	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	●	+	+					Cucurbitaceae		
<i>Phaseolus vulgaris</i>	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+	●	+					Fabaceae		
<i>Cistus salviaefolius</i>	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	●					Cistaceae		
<i>Celastrus scandens</i>	26	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	●		+		Celastraceae		
<i>Impatiens balsamina</i>	27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●			Balsaminaceae	
<i>Malva sylvestris</i>	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	●			Malvaceae
<i>Abroma augusta</i>	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●		Sterculiaceae
<i>Rhamnus catharticus</i>	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	Rhamnaceae

TABLE 4. Continued.

		b																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
<i>Phyllanthus calycinus</i>	1	●																			Euphorbiaceae
<i>Linum usitatissimum</i>	2	-	●	+	+	+			+					+							Linaceae
<i>Pimelea ligustrina</i>	3	-	+	●	+	+	+	+							+	+					Thymelaeaceae
<i>Pteroceltis tatarinowii</i>	4	-	+	+	●	○		+		+											Ulmaceae
<i>Celtis occidentalis</i>	5	-	+	+	○	●	+	+		+	+		○								Ulmaceae
<i>Passiflora caerulea</i>	6	-	-	+	-	+	●	+	+	+											Passifloraceae
<i>F. cataphracta</i>	7	-	-	+	+	+	+	●	+	+	+	+	+	+	+						Flacourtiaceae
<i>Ficus carica</i>	8	-	+	-	-	-	+	+	●	+	+	○	○								Moraceae
<i>Cneorum tricoccon</i>	9	-	-	-	+	+	+	+	+	●	○	+	+	+	○						Cneoraceae
<i>C. pulverulentum</i>	10	-	-	-	-	+	-	+	+	○	●	+	+	+							Cneoraceae
<i>Daphne mezereum</i>	11	-	-	-	-	-	-	+	○	+	+	●	○	+	+	○					Thymelaeaceae
<i>Parietaria officinalis</i>	12	-	-	-	-	○	-	+	○	+	+	○	●	○	+	+			○		Urticaceae
<i>Urtica dioica</i>	13	-	+	-	-	-	-	+	-	+	+	+	○	●	+	+	+	+			Urticaceae
<i>Ptelea trifoliata</i>	14	-	-	+	-	-	-	+	-	○	-	+	+	+	●	+			+		Rutaceae
<i>Zanthoxylum alatum</i>	15	-	-	+	-	-	-	-	-	-	-	○	+	+	+	●	○				Rutaceae
<i>Citrus limon</i>	16	-	-	-	-	-	-	-	-	-	-	-	-	+	-	○	●	+			Rutaceae
<i>Hydnocarpus alpina</i>	17	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	●	+		Flacourtiaceae
<i>Laportea canadensis</i>	18	-	-	-	-	-	-	-	-	-	-	-	○	-	+	-	-	+	●	+	Urticaceae
<i>Toxicodendron verni- ciflua</i>	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	●	Anacardiaceae

thoideae, Acalyphoideae, Crotonoideae, and Euphorbioideae are discussed.

Phyllanthoideae. Reference genus: *Phyllanthus*.

Tested genera: *Bridelia* (tribe Brideliaceae, according to Webster's treatment in this issue, 1994), *Antidesma*, *Baccaurea* (tribe Antidesmeae), *Drypetes*, *Putranjiva* (tribe Drypeteae), *Andrachne*, *Breynia*, *Glochidion*, *Margaritaria*, *Phyllanthus*, *Reverchonia*, *Securinega* (all tribe Phyllanthae).

Only seven genera included in the cross-reaction experiments with the *Phyllanthus* reference system (Fig. 1a) will be discussed. *Reverchonia*, *Breynia*, *Andrachne*, and *Securinega* are serologically the most similar genera to *Phyllanthus* among the tested taxa, in agreement with the inclusion of these four taxa within the tribe Phyllanthae. *Margaritaria* is less similar, and the double spurs with the precipitation of *Homalanthus*, *Sapium*, and *Hura* (Table 1a) can be interpreted in terms of overlapping similarity with *Phyllanthus*. *Antidesma* (tribe Antidesmeae) was found to be taxonomically and serologically different from the genera included in the Phyllanthae.

Acalyphoideae. Reference genus: *Ricinus*.

Tested genera: *Chrozophora* (tribe Chrozophoreae), *Mallotus*, *Mercurialis*, *Ricinus* (all tribe Acalypheae), *Dalechampia* (tribe Plukenetieae), *Omphalea* (tribe Omphaleae), *Adelia* (tribe Adeliaceae).

The serological data (Table 1b, Fig. 1b) indicate close similarity between *Mercurialis* and *Ricinus* (both tribe Acalypheae). However, the affinities of *Chrozophora* (tribe Chrozophoreae) to *Ricinus* still exceed those of *Mercurialis*. The data reveal a strong legumin similarity between all three genera, which does not agree with the present tribal arrangements. Since the data from the *Hura* and *Hevea* reference systems also reveal decreasing affinities in the sequence *Ricinus*-*Chrozophora*-*Mercurialis*, the three genera should be phylogenetically connected to non-Acalyphoideae subfamilies through *Ricinus*.

Mallotus, although included in the tribe Acalypheae, demonstrates only a distant relationship to *Ricinus*. *Dalechampia* (tribe Plukenetieae) has a different legumin, which is in agreement with the separate tribal position. Both *Mallotus* and *Dalechampia* are connected with the *Reutealis* (Crotonoideae) reference system (Fig. 4).

Crotonoideae. Reference genera: *Hevea*, *Reutealis*.

Tested genera: *Hevea* (tribe Micrandreae), *Manihot* (tribe Manihoteae), *Jatropha*, *Joannesia*

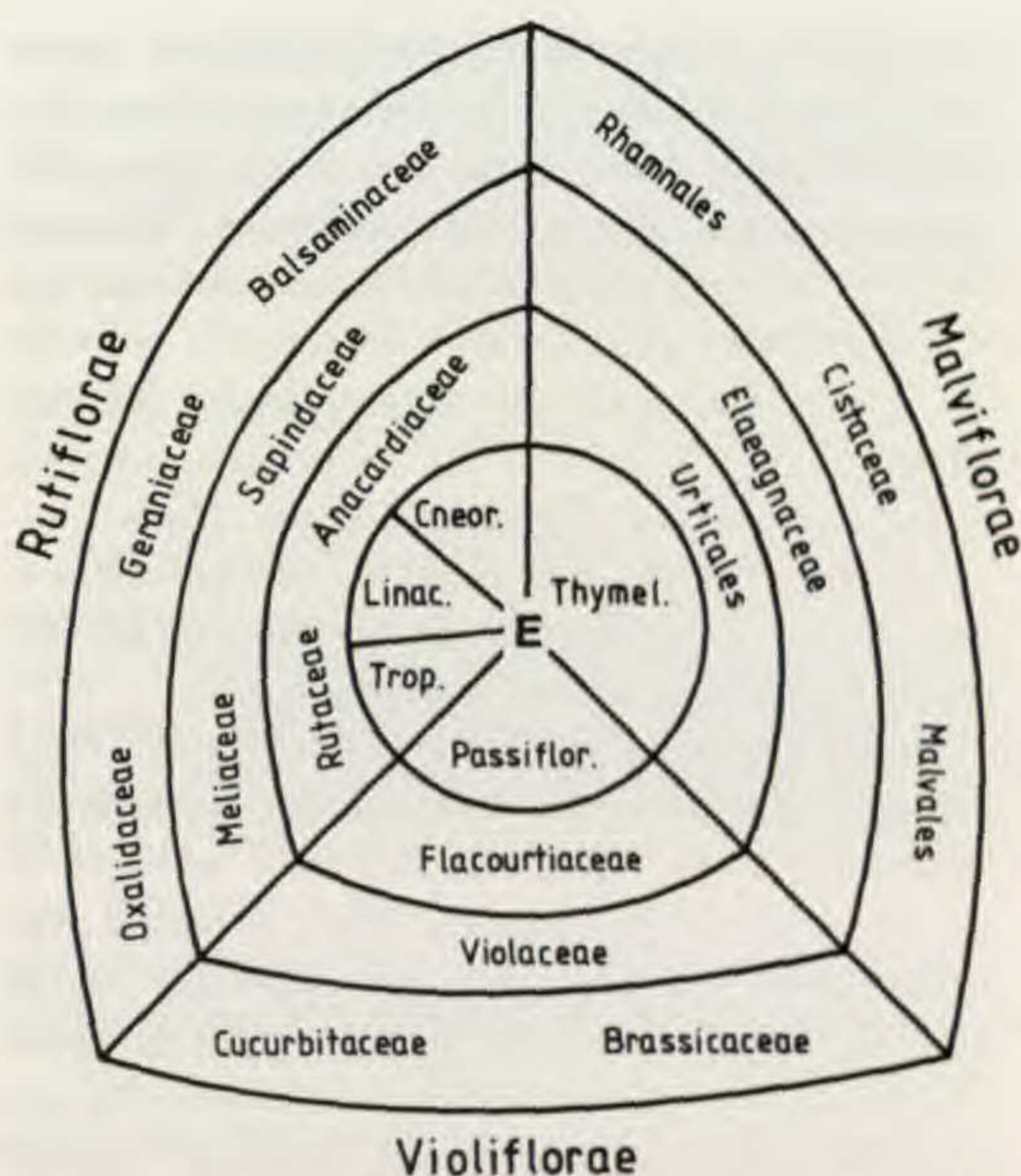


FIGURE 5. Serological similarities between the Euphorbiaceae (E) and outgroup families or orders of the Rutiflorae, Malviflorae, and Violiflorae. Similarity index decreases progressively from E toward the periphery.

(both tribe Jatropheae), *Strophoblachia* (tribe Codieae), *Aleurites*, *Reutealis*, *Vernicia* (all tribe Aleuritideae), *Croton*, *Eremocarpus* (tribe Crotonae).

The relationships of taxa included in the Crotonoideae were tested with two reference systems. They demonstrate a great phylogenetic complexity for this subfamily. From the comparison with the *Hevea* reference system (tribe Micrandreae) (Table 1c, Fig. 1c), the high serological correspondence with *Manihot* (tribe Manihoteae) and—although of less degree—*Croton* (tribe Crotonae) can be interpreted as supporting close relationships. However, the previous statement has to be kept in mind when interpreting the high serological correspondence with taxa from other subfamilies, i.e., *Ricinus* (Acalyphoideae), *Homalanthus* and *Hura* (both Euphorbioideae). The close relationship of *Jatropha* and *Joannesia* was not confirmed; *Jatropha* is loosely connected to the tribe Aleuritideae, whereas *Joannesia* is connected to *Hevea*, *Ricinus*, and *Hura* (Table 1c; Figs. 1c, 3). *Strophoblachia* (tribe Codieae) was employed in the experiments with the *Phyllanthus* reference system only, where it is close to *Joannesia*.

Aleurites, *Reutealis*, and *Vernicia* are all members of the tribe Aleuritideae subtribe Aleuritinae. They produce a similar legumin, which establishes their close relationship. The significance of this

statement is supported by the prominent cross-reactivities in the *Reutealis* reference system (Fig. 1d) and similar cross-reactivities in the other reference systems. The distant position of *Hevea* in this reference system is congruent with the concept of considerable phylogenetic differences between the Aleuritideae and the *Hevea-Hura-Ricinus* groups.

Euphorbioideae. Reference genus: *Hura*.

Tested genera: *Excoecaria*, *Homalanthus*, *Sapium* (all tribe Hippomaneae), *Hura* (tribe Hureae), *Euphorbia* (tribe Euphorbieae).

In this serological experiment *Excoecaria*, *Sapium*, and *Homalanthus* demonstrated structurally similar legumins, which support their inclusion in the same tribe Hippomaneae. They revealed close connections to *Hura* and also, especially in the case of *Homalanthus*, to the Crotonoideae genus *Hevea*. *Euphorbia* has no close serological affinities to either the Euphorbioideae reference system (*Hura*), nor to any other reference system used. Its major similarities are directed toward the *Reutealis* reference system. These reactions demonstrate the derived position of *Euphorbia* with respect to the molecular properties investigated, which is consistent with morphological characteristics.

RELATIONSHIPS BETWEEN EUPHORBIACEAE AND OTHER ANGIOSPERMS

Taxonomic and phylogenetic discussions on the relationships of the Euphorbiaceae within the angiosperms (see Webster, 1987) have suggested a common origin that favors the Malviflorae, Violiflorae, or Rutiflorae (Baillon, 1858; Bentham, 1878; Corner, 1976; Croizat, 1973; Dahlgren, 1983; Huber, 1985; Hutchinson, 1926; Lindley, 1836, 1853; Mueller, 1866; Pax, 1890, 1924; Takhtajan, 1969, 1980; Thorne, 1983; Webster, 1967; Wettstein, 1935). It is significant that data from our research, based upon the legumin properties, support the same systematic conclusions (see Table 3).

Although serological and morphological characters connect the Euphorbiaceae with Malviflorae, Violiflorae, or Rutiflorae, this does not apply to all taxa of the orders equally.

Malviflorae. Dahlgren (1980, 1983), Dahlgren et al. (1981), Takhtajan (1980), Thorne (1976, 1981), and many other authors regarded Euphorbiaceae and the order Malvales as related. The Rhamnales have been included in the discussions of relationship (Baillon, 1858; Bentham, 1878, 1880; Cronquist, 1981; Hutchinson, 1969; Lindley, 1853; Stebbins, 1974). However, neither the Malvales nor the Rhamnales have been found to

be serologically related to the Euphorbiaceae. Therefore, we believe that phylogenetic relationships between Euphorbiaceae and Malvales, as well as between Euphorbiaceae and Rhamnales, are unlikely.

In contrast to the orders previously discussed, the Urticales (Moraceae, Urticaceae, Ulmaceae) prove to be serologically closely allied with the Euphorbiaceae. The serological characters in favor of these close phylogenetic relationships are supported by the following characters: reduced, often unisexual flowers and a hypogynous gynoecium containing a bitegmic and crassinucellate ovule in both the Euphorbiaceae and Urticales and the occurrence of laticifers in Euphorbiaceae and Moraceae. Additionally, chemical similarities (alkaloids, accumulation of calcium oxalate and silica) support the relationships between Euphorbiaceae and Urticales. Such evaluations have influenced Berg (1977), Dahlgren (1983), and Thorne (1976) to hypothesize close relationships between these two taxonomic groups.

The serological similarities between Euphorbiaceae and Urticales are exceeded by the similarities between Euphorbiaceae and Thymelaeaceae. Toxic phorbolic esters are produced in these two families (Frohne & Jensen, 1992). A crotonoid pollen exine and an exotegmic seed coat with a palisade layer derived from the outer epidermis of the inner integument (Wunderlich, 1968; Corner, 1976) are additional common characteristics. These characters confirm the close relationships between the two families. Several classifications included these families in the single order Euphorbiales to reflect this similarity (e.g., Frohne & Jensen, 1992; Thorne, 1968, 1983).

Violiflorae. Legumin antigens extracted from seeds of Cucurbitaceae and Brassicaceae species cross-reacted only weakly with Euphorbiaceae antisystems (references: *Phyllanthus* and *Ricinus*). However, members of the Violales, especially Flacourtiaceae and Passifloraceae, for which serological seed properties different from Cucurbitaceae have been reported by Kolbe & John (1979), share a relatively high portion of serological characters with the Euphorbiaceae. In cross-reactivity they exceed many Euphorbiaceae taxa. The flowers of Violales are characterized by, among other characters, three carpels, a hypogynous gynoecium, and bitegmic and crassinucellate ovaries. All these characters distinguish Euphorbiaceae flowers. Takhtajan (1969), Corner (1976), and Huber (1985) have regarded both groups as related. In this context the Flacourtiaceae have proven to be of interest because this family is regarded as es-

pecially closely connected to the phylogenetic origin of the Violales. Passifloraceae revealed the highest serological correspondence to the Euphorbiaceae reference systems of *Ricinus* (Table 4a) and also of *Phyllanthus* (Fig. 4b). A valvate calyx, starchless endosperm, straight embryo, and an extrastaminal floral disk are characteristic properties of the Passifloraceae, and these characters in addition to the seed protein data reinforce the theory of a close relationship between Violales and Euphorbiaceae.

Rutiflorae. Many Rutiflorae species included in our serological experiments revealed important positive cross-reactions. These cross-reactions demonstrated legumin similarities, with the Euphorbiaceae reference systems (*Phyllanthus* and *Ricinus*) being in the same range as the cross-reactions of many Euphorbiaceae species. However, the Balsaminaceae (Balsaminales), in contrast to the Linaceae (Geraniales), failed significantly to react as well as Oxalidaceae and Geraniaceae (both Geraniales). The following three Rutiflorae families revealed especially high legumin correspondence with the Euphorbiaceae: Tropaeolaceae, Cneoraceae, Linaceae.

The ovaries of the Tropaeolaceae fall apart into three cocci upon maturity, as do those of the Euphorbiaceae. They also possess an extrastaminate flower disk and stipulate leaves. Chemically, the production of glucosinolates is noteworthy because this is also reported for the Euphorbiaceae taxa *Putranjiva roxburghii* Wall. and *Jatropha multifida* L. (Rizk, 1987). In this connection it is important to recall that no serological similarities have been detected between the glucosinolate-producing Brassicaceae and the Tropaeolaceae (Jensen, 1991; Kolbe, 1978) or between Brassicaceae and Euphorbiaceae (Jensen, 1991).

For the Cneoraceae, the tricocous gynoecium, floral disks, hypogynous ovaries, and one or two pendulous ovules per carpel are diagnostic characters. These important morphological similarities indicate possible close phylogenetic relationships between Cneoraceae and Euphorbiaceae; however, these characters have not been utilized in formulating classifications.

In the relative placement series of legumin similarities the Linaceae occupy the first (reference: *Phyllanthus*) or second placement (reference: *Ricinus*) among the families tested, and have been emphasized when the relationships of the Euphorbiaceae are discussed. In addition to serological characters, other characters indicate significant similarities between the Linaceae and Euphorbiaceae that cannot be explained by convergence

alone. The Linaceae seeds produce copiously fatty oils and storage proteins, and are characterized by a hypogynous ovary, one or two bitegmic ovules for each carpel, an obturator, intra- or extrastaminal floral disks, and stipulate leaves.

ARE THE DILLENIIDAE AND ROSIDAE SUBCLASSES NATURAL GROUPS?

Our discussion of the relationships of the Euphorbiaceae indicates significant similarities between Euphorbiaceae and Malviflorae (especially Urticales and Thymelaeaceae), Violiflorae (especially Flacourtiaceae and Passifloraceae), and Rutiflorae (especially Tropaeolaceae, Cneoraceae, and Linaceae) (Fig. 5). The corresponding morphological similarities have been reported by several authors since Baillon's (1858) publication.

Thus the Euphorbiaceae are possibly closely related to the Dilleniidae (i.e., Malviflorae, Violiflorae) as well as to the Rosidae (i.e., Rutiflorae), which are two distinct subclasses in many classifications (e.g., Cronquist, 1981; Takhtajan, 1987). Therefore, the separation of the Dilleniidae and Rosidae subclasses has to be questioned and a taxonomic revision of the Dilleniidae-Rosidae-complex is considered necessary. Within this large collection of orders in the "Mittelbau" (Ehrendorfer, 1991) of the angiosperms, there is a group of families that show similar characters, i.e., stipulate leaves, reduced and sometimes unisexual flowers, floral disks, trimeric and hypogynous gynoecia with two or one ovules per carpel, bitegmic and crassinucellate ovules with obturator, laticifers, and cyanogenic and glucosinolate compounds. In this taxonomic complex, the Euphorbiaceae are included.

LITERATURE CITED

- BAILLON, H. 1858. *Etude Générale du Groupe des Euphorbiacées*. Victor Masson, Paris.
- BENTHAM, G. 1878. Notes on Euphorbiaceae. *J. Linn. Soc., Bot.* 17: 185-267.
- . 1880. Euphorbiaceae. In: G. Bentham & J. D. Hooker (editors), *Genera Plantarum* 3: 239-340.
- BERG, C. C. 1977. Urticales, their differentiation and systematic position. *Pl. Syst. Evol., Suppl.* 1: 349-374.
- BERGNER, I. & U. JENSEN. 1989. Phytoserological contribution to the systematic placement of the Typhales. *Nordic J. Bot.* 8: 447-456.
- CORNER, E. J. H. 1976. *The Seeds of Dicotyledons*. 2 vols. Cambridge Univ. Press, Cambridge.
- CRISTOFOLINI, G. 1980. Interpretation and analysis of serological data. Pp. 269-288 in F. A. Bisby, J. G. Vaughan & D. Wright (editors), *Chemosystematics. Principles and Practice*. Academic Press, London and New York.
- CROIZAT, L. 1973. Les Euphorbiacées vues en elles-

- mêmes, et dans leurs rapports envers l'angiospermie en général. *Mem. Soc. Brot.* 23: 5-206.
- CRONQUIST, A. 1981. *An Integrated System of Classification of Flowering Plants*. Columbia Univ. Press, New York.
- DAHLGREN, R. 1980. A revised system of classification of the angiosperms. *Bot. J. Linn. Soc.* 80: 91-124.
- . 1983. General aspects of angiosperm evolution and macrosystematics. *Nordic J. Bot.* 3: 119-149.
- , S. R. JENSEN & B. J. NIELSEN. 1981. A revised classification of the angiosperms with comments on correlations between chemical and other characters. Pp. 149-199 in D. A. Young & D. S. Seigler (editors), *Phytochemistry and Angiosperm Phylogeny*. Praeger, New York.
- EHRENDORFER, F. 1991. Samenpflanzen. Pp. 699-828 in P. Sitte, H. Ziegler, F. Ehrendorfer & A. Bresinsky (editors), *Lehrbuch der Botanik für Hochschulen*. G. Fischer Verlag, Stuttgart, New York.
- FAIRBROTHERS, D. E. 1977. Chemosystematics. *Bull. Serol. Mus.* 52: 3.
- . 1983. Evidence from nucleic acid and protein chemistry, in particular serology, in angiosperm classification. *Nordic J. Bot.* 3: 35-41.
- , T. J. MABRY, R. L. SCOGIN & B. L. TURNER. 1975. The bases of angiosperm phylogeny: Chemotaxonomy. *Ann. Missouri Bot. Gard.* 62: 765-800.
- FISCHER, H. & U. JENSEN. 1992. Utilization of proteins to estimate relationships in plants: Serology. A discussion based on the Asteraceae-Cichorioideae. *Belg. J. Bot.* 125: 243-255.
- FROHNE, D. & U. JENSEN. 1992. *Systematik des Pflanzenreichs*, 4. Aufl. G. Fischer Verlag, Stuttgart, New York.
- HUBER, H. 1985. *Angiospermen. Leitfaden durch die Ordnungen und Familien der Bedecktsamer*. Gustav Fischer Verlag, Stuttgart, New York.
- HUTCHINSON, J. 1926. *The Families of Flowering Plants I. Dicotyledons*. Macmillan, London.
- . 1969. *Evolution and Phylogeny of Flowering Plants*. Academic Press, London.
- JENSEN, U. 1984. Legumin-like and vicilin-like storage proteins in *Nigella damascena* (Ranunculaceae) and six other dicotyledonous species. *J. Pl. Physiol.* 115: 161-170.
- . 1991. Steps toward the natural system of the dicotyledons: Serological characters. *Aliso* 13: 183-190.
- & H. BERTHOLD. 1989. Legumin-like proteins in gymnosperms. *Phytochemistry* 28: 1389-1394.
- & C. BÜTTNER. 1981. The distribution of storage proteins in Magnoliophytina (angiosperms) and their serological similarities. *Taxon* 30: 404-419.
- & D. E. FAIRBROTHERS (editors). 1983. *Proteins and Nucleic Acids in Plant Systematics*. Springer-Verlag, Berlin, Heidelberg.
- & B. GREVEN. 1984. Serological aspects and phylogenetic relationships of the Magnoliidae. *Taxon* 33: 563-577.
- & B. GRUMPE. 1983. Seed storage proteins. Pp. 238-254 in U. Jensen & D. E. Fairbrothers (editors), *Proteins and Nucleic Acids in Plant Systematics*. Springer-Verlag, Berlin, Heidelberg.
- KOLBE, K.-P. 1978. Serologischer Beitrag zur Systematik der Capparales. *Bot. Jahrb. Syst.* 99: 468-489.
- & J. JOHN. 1979. Serologische Untersuchungen zur Systematik der Violales. *Bot. Jahrb. Syst.* 101: 3-15.
- LESTER, R. N., P. A. ROBERTS & C. LESTER. 1983. Analysis of immunotaxonomic data obtained from spur identification and absorption techniques. Pp. 275-300 in U. Jensen & D. E. Fairbrothers (editors), *Proteins and Nucleic Acids in Plant Systematics*. Springer-Verlag, Berlin, Heidelberg.
- LINDLEY, J. 1836. *A Natural System of Botany*. London.
- . 1853. *The Vegetable Kingdom*, 3rd ed. Bradbury & Evans, London.
- MUELLER, J. 1866. Euphorbiaceae. In: A. P. de Candolle (editor), *Prodromus Systematis Naturalis Regni Vegetabilis* 15: 189-1261. Masson, Paris.
- PAX, F. 1890. Euphorbiaceae. In: A. Engler & K. Prantl (editors), *Die Natürlichen Pflanzenfamilien*. Erste Auflage, III. 3: 1-119. W. Engelmann, Leipzig.
- . 1924. Die Phylogenie der Euphorbiaceae. *Bot. Jahrb. Syst.* 59: 129-182.
- RIZK, A.-F. M. 1987. The chemical constituents and economic plants of the Euphorbiaceae. Pp. 293-326 in S. L. Jury, T. Reynolds, D. F. Cutler & F. J. Evans (editors), *The Euphorbiales. Chemistry, Taxonomy and Economic Botany*. Academic Press, London.
- ROHLF, F. J. 1989. NTSYS-pc; Numerical Taxonomy and Multivariate Analysis System. Exeter Publishing, Setauket, New York.
- SCHULTES, R. E. 1987. A new generic concept in the Euphorbiaceae. *Bot. Mus. Leaflet* 17: 27-36.
- SNEATH, P. H. A. & R. R. SOKAL. 1973. *Numerical Taxonomy*. Freeman, San Francisco.
- STEBBINS, G. L. 1974. *Flowering Plants: Evolution Above the Species Level*. Belknap Press, Harvard Univ., Cambridge, Massachusetts.
- TAKHTAJAN, A. 1969. *Flowering Plants, Origin and Dispersal*. Oliver & Boyd, Edinburgh.
- . 1980. Outline of the classification of flowering plants (Magnoliophyta). *Bot. Rev. Lancaster* 46: 225-359.
- . 1987. *Systema Magnoliophytorum*. Nauka, Leninopoli. [In Russian.]
- THORNE, R. F. 1968. Synopsis of a putatively phylogenetic classification of the flowering plants. *Aliso* 6: 57-66.
- . 1976. A phylogenetic classification of the Angiospermae. *Evol. Biol.* 9: 35-106.
- . 1981. Phytochemistry and angiosperm phylogeny: A summary statement. Pp. 233-295 in D. A. Young & D. S. Seigler (editors), *Phytochemistry and Angiosperm Phylogeny*. Praeger, New York.
- . 1983. Proposed new realignments in the angiosperms. *Nordic J. Bot.* 3: 85-117.
- VOGEL, CH. 1986. *Phytoserologische Untersuchungen zur Systematik der Euphorbiaceae*. *Dissertationes Botanicae*, 98. J. Cramer, Berlin, Stuttgart.
- WEBSTER, G. L. 1967. The genera of Euphorbiaceae in the south-eastern United States. *J. Arnold Arboretum* 48: 303-430.
- . 1975. Conspectus of a new classification of the Euphorbiaceae. *Taxon* 24: 593-601.
- . 1987. The saga of the spurges: A review of classification and relationships in the Euphorbiales.